Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 4 pp. 351–356, 2009 ISSN 0001-6837
Polish Pharmaceutical Society

VALIDATION OF DERIVATIVE SPECTROPHOTOMETRY METHOD FOR DETERMINATION OF ACTIVE INGREDIENTS FROM NEUROLEPTICS IN PHARMACEUTICAL PREPARATIONS

MARIUSZ STOLARCZYK , ANNA APOLA, JAN KRZEK AND ANNA SAJDAK

Department of Inorganic and Analytical Chemistry, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland

Abstract: First (D1) and second (D2) order derivative spectrophotometric method with an application of base line to peak technique was used for determination of active pharmaceutical ingredients (API) at two wavelengths: fluphenazine (D1 at λ = 251 nm and λ = 265 nm, D2 at λ = 246 nm and λ = 269 nm), pernazine (D1 at λ = 246 nm and λ = 258 nm, D2 at λ = 254 nm and λ = 262 nm), haloperidol (D1 at λ = 235 nm and λ = 253 nm, D2 at λ = 230 nm and λ = 246 nm), and promazine (D1 at λ = 246 nm and λ = 251 nm, D2 at λ = 255 nm and λ = 262 nm). Linear dependence of derivative values on analyte concentration is maintained in a range 3.12 $\mu g \times mL^{-1}$ – 44.80 $\mu g \times mL^{-1}$. Detection and determination limits are in the range 0.51 – 3.23 $\mu g \times mL^{-1}$ and 1.27 $\mu g \times mL^{-1}$ – 9.80 $\mu g \times mL^{-1}$, respectively. Determination results of drug constituents are very accurate. Recovery percentage is in a range 95.50% – 103.60%.

Keywords: derivative spectrophotometry, fluphenazine**,** pernazine, haloperidol, promazine, drug analysis

Neuroleptic drugs are much diversified on the basis of chemical constitution. Derivatives of phenotiazine, such as fluphenazine (2-[4-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-piperazin-1-yl]ethanol), perazine (10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine), promazine (N,N-dimethyl-3-(10H-phenothiazin-10-yl) propane-1-amine), and derivatives of butyrophenone, such as haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one), are classified within this group of drugs.

Due to a huge diversity of chemical structures in studied group of drugs, a lot of analytical methods adapted for determination of active pharmaceutical ingredients in pharmaceutical preparations as well as body fluids could be found in published literature. Among the most frequently used techniques in analysis of phenotiazine derivatives are: high-performance liquid chromatography (HPLC) (1-5), liquid chromatography (LC) (6, 7), gas chromatography (GC) (8, 9) and thin-layer chromatography (TLC) (10). The HPLC (11, 12) and mass spectrometry coupled with liquid chromatography (LC–MS) (13) methods are used in analysis of metabolites of haloperidol in biological materials, whereas high-performance thin-layer chromatography method (HPTLC) (14) is used for analysis of haloperidol in pharmaceutical formulations (15).

Studies were done to develop and validate new derivative spectrophotometry method for determination of fluphenazine (FL), pernazine (PE), haloperidol (HL) and promazine (PR) in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

Spectrophotometer: UV-VIS Cary 100 (Varian), quartz cuvettes (l = 1 cm). Computer: PC Dell Optiplex 755; Brother HL-1430; printer and software (Microsoft Windows XP 2002; Statistica 8.0).

Standard solutions

Solutions were diluted with methanol to obtain concentrations in a range 8.96 $\mu g \times mL^{-1}$ to 44.80 $\mu g \times mL^{-1}$ (fluphenazine), 5.10 $\mu g \times mL^{-1}$ to 22.50 $\mu g \times mL^{-1}$ (pernazine), 4.56 $\mu g \times mL^{-1}$ to 22.82 $\mu g \times mL^{-1}$ (haloperidol), and 3.12 $\mu g \times mL^{-1}$ –to 15.60 $\mu g \times mL^{-1}$ (promazine) for direct analyses.

* Corresponding author: mstolar@cm-uj.krakow.pl

Sample solutions

Fluphenazine: an amount of 95.40 mg -118.20 mg was weighed from powdered mass of 10 tablets of Mirenil formulation (Jelfa, Poland) with accuracy up to 0.1 mg. The weighed amount was poured with 10 mL of methanol and shaked for 15 min, followed by centrifugation at 1500 rpm.

Pernazine: an amount of 71.10 mg – 82.40 mg was weighed from powdered mass of 10 tablets of Pernazinum formulation (Hasco-Lek, Poland) with accuracy up to 0.1 mg. The weighed amount was poured with 10 mL of methanol (1 mol×L^{-1}/water, at ratio 1:1) and shaked for 15 min, followed by centrifugation at 1500 rpm.

Haloperidol: an amount of 100.80 mg – 142.10 mg was weighed from powdered mass of 10 tablets of Haloperidol formulation (Polfa Warsaw, Poland) with accuracy up to 0.1 mg. The weighed amount was poured with 10 mL of methanol and shaked for 15 min, followed by centrifugation at 1500 rpm.

Promazine: an amount of 45.80 mg – 57.90 mg was weighed from powdered mass of 10 tablets of Promazine formulation (Jelfa, Poland) with accuracy up to 0.1 mg. The weighed amount was poured with 10 mL of methanol and shaked for 15 min, followed by centrifugation at 1500 rpm.

A volume of 100 mL of each studied solution was taken and filled up with methanol to a total volume of 10.0 mL for direct analysis.

RESULTS AND DISCUSSION

In the first phase of study, zero-order absorption spectra were recorded at 200 nm – 400 nm for standard solutions containing studied active pharmaceutical ingredients. Methanol was used as a blank test and spectrophotometric measurements were done. The results are presented in Figure 1.

The analysis of zero-order spectrum allows for observation of very similar spectrum of promazine, pernazine and fluphenazine. Absorption maxima for all three substances are in a range 253 – 260 nm. Haloperidol has slightly diversified spectrum with two maxima at 221 nm and 243 nm (Fig. 1).

Conversion of zero-order spectra into D1 and D2 derivatives caused their significant diversification with reference to very well-developed absorption maxima occurring at various wavelengths. Two wavelengths for each studied standard solution were chosen during analysis of D1 and D2 derivative curves by the base line to peak technique. The content of fluphenazine (FL) was determined at wavelengths $\lambda_{251.0\ nm}$ and $\lambda_{265.0\ nm}$ for the first derivative (D1) and $\lambda_{246.0\ nm}$ and $\lambda_{269.0\ nm}$ for the second derivative (D2) (Fig. 2).

Similarly, the analysis was done for pernazine (PE) at $\lambda_{246.0\ nm}$, $\lambda_{258.0\ nm}$ (D1) and $\lambda_{254.0\ nm}$, $\lambda_{262.0\ nm}$ (D2) (Fig. 3), haloperidol (HL) at $\lambda_{235.0\ nm}$, $\lambda_{253.0\ nm}$

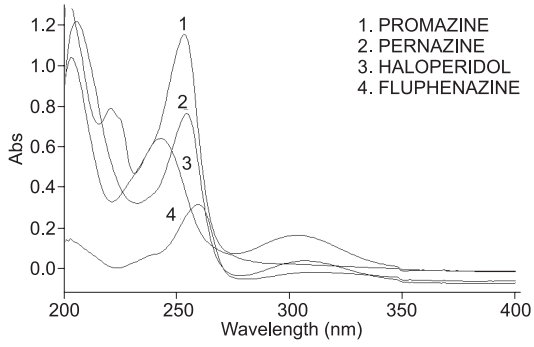

Figure 1. The zero order absorption spectra for examined substances.

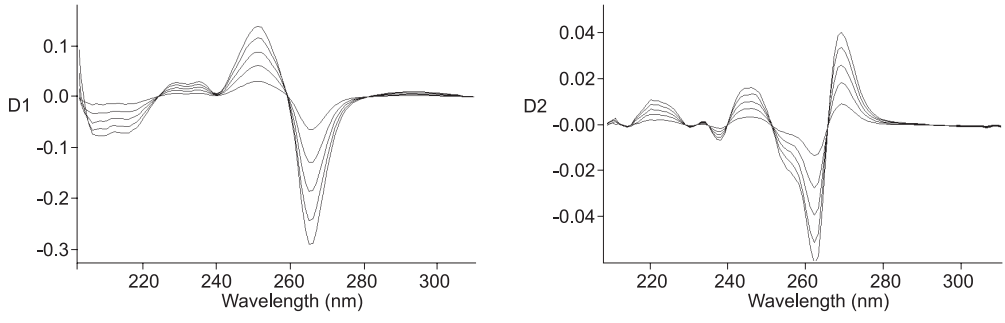

Figure 2. The first D1 and second D2 derivative spectra for standard solutions of fluphenazine. Concentrations: 8.96, 17.92, 26.88, 35.84, 44.80 µg×mL^{-1}.

(D1) and $\lambda_{230.0\ nm}$, $\lambda_{246.0\ nm}$ (D2) (Fig. 4) and promazine (PR) at $\lambda_{246.0\ nm}$, $\lambda_{259.0\ nm}$ (D1) and $\lambda_{255.0\ nm}$, $\lambda_{262.0\ nm}$ (D2) (Fig. 5).

Validation of the method regarding specificity, linearity, detection and determination limits was done in another phase of the study.

Specificity

Due to a lack of data concerning composition of placebo, comparative studies were done for model solutions (R_m) and ready-to-use pharmaceutical forms which were the issue of studies (P_f). Solutions with comparable content, i.e. containing 75%, 100% and 125% individual constituents were prepared for analysis. Measurements of the derivative values were done at the chosen wavelengths. Specificity of the method for analyte was determined by comparing some derivative values of model solutions and pharmaceutical formulations.

Comparable values of some derivatives, according to dependence $\frac{R_m}{P_f}$, do not differ significantly from 1, and are in the range 0.96–1.05. This testifies for almost identical derivative curves of model solutions and pharmaceutical formulations. Based on these results, it can be concluded that placebo that was used for formulation of an adequate drug form does not affect quantity analysis of an active pharmaceutical ingredient.

Linearity

To check the linearity, five measurements of standard solutions were done in the ranges (Fig. 4–5): from 8.96 $\mu g \times mL^{-1}$ to 44.80 $\mu g \times mL^{-1}$ (for fluphenazine). from 5.10 $\mu g \times mL^{-1}$ to 25.50 $\mu g \times mL^{-1}$ (for pernazine), from 4.56 $\mu g \times mL^{-1}$ to 22.82 $\mu g \times mL^{-1}$ for haloperidol and from 3.12 $\mu g \times mL^{-1}$ to 15.60 $\mu g \times mL^{-1}$ (for promazine).

Linearity is maintained in the studied ranges of concentrations. Linear regression equations with points of intersection of straight line and correlation coefficients were applied for calculations of results (Table 1).

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated, using the values of statistical parameters for adequate calibration curves according to equations: $LOD = 3.3 \cdot S_Y/a$, and $LOQ = 10.0 \cdot S_Y/a$, where S_Y – standard error of the estimate, a – slope of a straight line coefficient (Table 2).

Recovery

Recovery for individual constituents was presented in percentage on the basis of determination of analyte concentration that was added in the amount of 80% – 120% of declared amount to test samples. The determination results together with statistical calculation of an average value ($x_{aver.}$),

Table 1. Wavelengths, regression equations and corelation cofficients for standard solutions of examined substances.

Fluphenazine	λ = 251.0nm	D1 = 0.00631 + 0.00298×c	r = 0.99875
	λ = 265.0nm	D1 = 0.01203 + 0.00626×c	r = 0.99842
	λ = 246.0nm	D2 = 0.00058 + 0.00035×c	r = 0.99849
	λ = 269.0nm	D2 = 0.00241 + 0.00086×c	r = 0.99821
Pernazine	λ = 246.0nm	D1 = 0.000045 + 0.00217×c	r = 0.99986
	λ = 258.0nm	D1 = -0.0007 + 0.00507×c	r = 0.99986
	λ = 254.0nm	D2 = -0.0005 + 0.00116×c	r = 0.99986
	λ = 262.0nm	D2 = -0.000001 + 0.00060×c	r = 0.99991
Haloperidol	λ = 253.0nm	D1 = -0.0001 + 0.00174×c	r = 0.99974
	λ = 235.0nm	D1 = 0.00014 + 0.0010×c	r = 0.99951
	λ = 230.0nm	D2 = 0.00032 + 0.00088×c	r = 0.99943
	λ = 246.0nm	D2 = -0.000004 + 0.00021×c	r = 0.99994
Promazine	λ = 246.0nm	D1 = 0.00270 + 0.00412×c	r = 0.99894
	λ = 259.0nm	D1 = 0.00314 + 0.00937×c	r = 0.99126
	λ = 255.0nm	D2 = 0.00095 + 0.0023×c	r = 0.99923
	λ = 269.0nm	D2 = 0.00038 + 0.00103×c	r = 0.99925

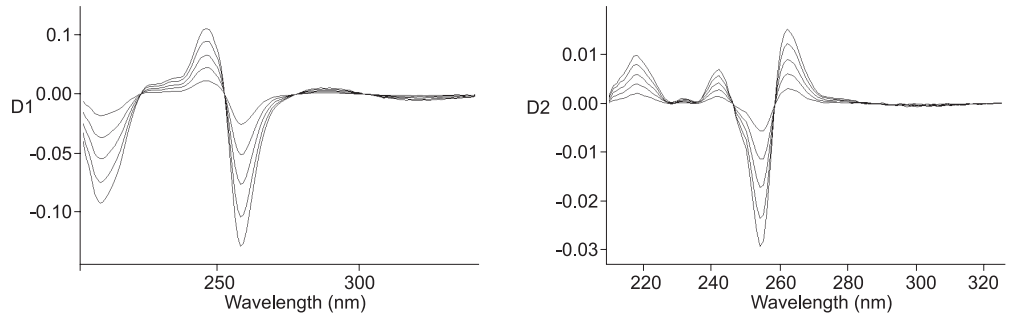

Figure 3. The first D1 and second D2 derivative spectra for standard solutions of pernazine. Concenrations: 5.10, 10.20, 15.30, 20.40, 25,50 µg×mL^{-1}.

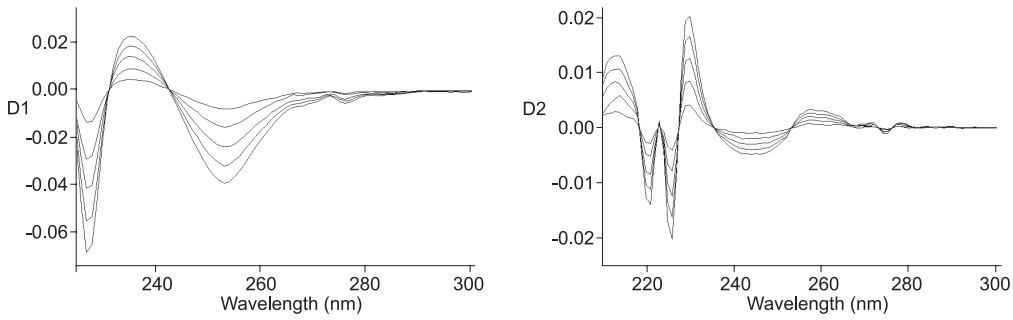

Figure 4. The first D1 and second D2 derivative spectra for standard solutions of haloperidol. Concentrations: 4.56, 9.13, 13.69, 18.26, 22.82 µg×mL^{-1}.

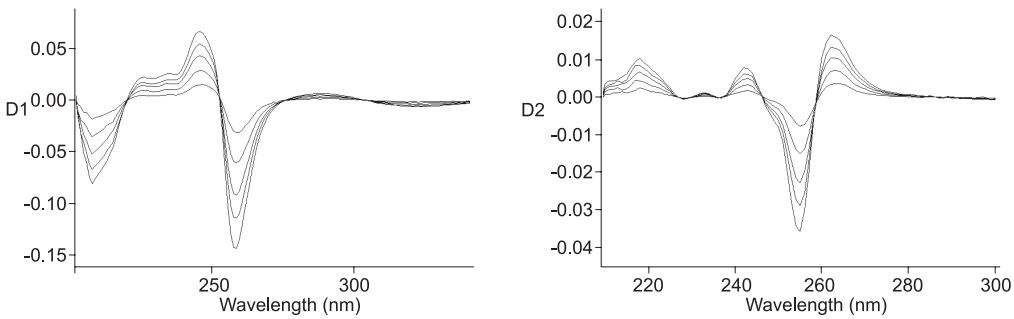

Figure 5. The first D1 and second D2 derivative spectra for standard solutions of promazine. Concentrations: 3.12, 6.24, 9.36, 12.48, 15.60 µg×mL^{-1}.

standard deviation (S_x), relative standard deviation (RSD%) and confidence interval ($t_{0.95}$) are presented in Table 3.

Determination procedure was developed on the basis of study results.

Quantitative analysis

Absorption spectra of standard solutions mixture of studied formulations and solutions of studied test samples were recorded at 200 nm – 400 nm. Zero-order spectra were converted to first-order (D1) and second-order (D2) derivatives. For quantity analyses, the values were read at chosen wavelengths with the use of D1 and D2 derivatives. A content of active pharmaceutical ingredients was calculated by comparison of adequate derivatives values of standard solution and studied trial.

Table 2. Determined values of LOD and LOQ

Substance	LOD [µg×mL^{-1}]				LOQ [µg×mL^{-1}]			
	$D1_{\lambda1}$	$D1_{\lambda2}$	$D2_{\lambda3}$	$D2_{\lambda4}$	$D1_{\lambda1}$	$D1_{\lambda2}$	$D2_{\lambda3}$	$D2_{\lambda4}$
Fluphenazine	3.03	1.28	3.01	3.23	9.20	8.20	9.11	9.80
Pernazine	0.51	0.51	0.51	0.42	1.55	1.55	1.54	1.27
Haloperidol	0.63	0.87	0.93	0.31	1.89	2.62	2.82	0.93
Promazine	0.86	2.50	0.73	0.73	2.26	7.60	2.23	2.21

Table 3. Recovery of the determined substances with statistical evaluation

Substance	Recovery [%]	Statistical evaluation
Fluphenazine n = 6	99.6 – 101.7	$\overline{x}$ = 100.58 $t_{0.95}$ = ± 0.7433 S_x = 0.7083
Pernazine n = 6	97.3 – 103.6	$\overline{x}$ = 100.60 $t_{0.95}$ = ± 2.4132 S_x = 2.2996
Haloperidol n = 6	96.0 – 106.3	$\overline{x}$ = 101.88 $t_{0.95}$ = ± 4.2391 S_x = 4.0395
Promazine n = 6	95.5 – 103.6	$\overline{x}$ = 99.80 $t_{0.95}$ = ± 3.1082 S_x = 2.9618

$\overline{x}$ – mean value, $t_{0.95}$ – confidence interval for 95% probability, S_x – standard deviation

Determination results of the contents of constituents with their statistical estimation are presented in Table 4.

CONCLUSIONS

Based on the results, a conclusion could be made that the first-order (D1) and second-order (D2) derivative spectrophotometric method allows for quantity analysis of active pharmaceutical ingredients occurring in Mirenil, Pernazinum, Haloperidol and Promazine formulations. Contrary to classical spectrophotometry, the developed method allows for broadening a range of determination with the use of various wavelengths.

This method is an analyte-specific for studied constituents at chosen wavelengths. No interference

Table 4. Determination results with their statistical evaluation

Pharmaceutical preparation (determined constituent)	Declared content [mg/tablet]	Determined content (mean, n = 10) [mg/tablet]		Statistical evaluation	
		D1	D2	D1	D2
MIRENIL (fluphenazine)	0.25	0.2583	0.2617	S_x = 0.0041 $t_{0.95}$ = ± 0.0043 $\%E_{rel}$ = 3.32 RSD = 1.60%	S_x = 0.0117 $t_{0.95}$ = ± 0.0123 $\%E_{rel}$ = 3.30 RSD = 4.50%
PERNAZINUM (pernazine)	100.0	99.70	99.98	S_x = 4.9766 $t_{0.95}$ = ± 5.2226 $\%E_{rel}$ = 0.28 RSD = 2.96%	S_x = 4.5706 $t_{0.95}$ = ± 4.80 $\%E_{rel}$ = 0.02 RSD = 2.71%
HALOPERIDOL (haloperidol)	1.0	1.0	0.99	S_x = 0.0163 $t_{0.95}$ = ± 0.0230 $\%E_{rel}$=0.00 RSD = 2.20%	S_x = 0.0210 $t_{0.95}$ = ± 0.02100 $\%E_{rel}$ = 1.00 RSD = 2.02%
PROMAZIN (promazine)	50.0	48.31	49.24	S_x = 1.2706 $t_{0.95}$ = ± 1.3334 $\%E_{rel}$ = 3.40 RSD = 2.63%	S_x = 1.2647 $t_{0.95}$ = ± 1.3272 $\%E_{rel}$ = 1.53 RSD = 2.57%

of matrix constituents was observed what proves the selectivity of this method. The linearity is maintained in a wide range of concentrations, i.e. 8.96 $\mu g \times mL^{-1}$ – 44.80 $\mu g \times mL^{-1}$ (FL), 5.10 $\mu g \times mL^{-1}$ – 25.50 $\mu g \times mL^{-1}$ (PE), 4.56 $\mu g \times mL^{-1}$ – 22.82 $\mu g \times mL^{-1}$ (HL) and 3.12 $\mu g \times mL^{-1}$ – 16.60 $\mu g \times mL^{-1}$ (PR), with good correlation for D1 and also depends on chosen wavelengths: r = 0.99875 – 0.99842 (FL), 0.99986 – 0.99986 (PE), 0.99974 – 0.99951 (HL), 0.99894 – 0.99126 (PR), and for D2: r = 0.99849 – 0.99821 (FL), 0.99986 – 0.99991 (PE), 0.99943 – 0.99994 (HL) and 0.99923 – 0.99925.

Intersection points of straight line of adequate calibration curves are close to zero. The sensitivity of the developed method is high and is 8.2 $\mu g \times mL^{-1}$ to 9.80 $\mu g \times mL^{-1}$ (FL), 1.27 $\mu g \times mL^{1}$ to 1.55 $\mu g \times mL^{-1}$ (PE), 1.89 $\mu g \times mL^{-1}$ to 2.82 $\mu g \times mL^{-1}$ (HL) and 2.23 $\mu g \times mL^{-1}$ to 2.62 $\mu g \times mL^{-1}$ (PR). Moreover, this method in a significant degree depends on the order of derivative and wavelength.

Recovery percentage of studied constituents, which is presented as average values for three concentration levels, is high and is contained within 99.6%–101.7% (FL), 97.3%–103.6% (PE), 96.0%–106.3% (HL) or 95.5%–103.6% (PR).

The determination results of contents of individual constituents are close to the declared values and have narrow confidence interval and small values of standard deviation (S_x), relative error ($\%E_{rel}$) and relative standard deviation (RSD).

The results obtained during studies, besides scientific value, have also practical value. Because of its simplicity and speed, the developed method can be useful for quality control of drugs and an option for commonly used expensive chromatographic methods.

This paper is an example of a broader application of derivative spectrophotometry for the analysis of fluphenazine, pernazine, haloperidol and promazine. Besides all above-mentioned advantages, it is a low-cost and easily accessible method.

REFERENCES

1. Pistos C., Stewart J.T.: Biomed. Chromatogr. 17, 465 (2003).
2. Tanaka E., Nakamura T., Terada M., Shinozuka T., Hashimoto C., Kurihara K., Honda K.: J. Chromatogr. B 854, 116 (2007).
3. Luo J.P., Hubbard J.W., Midha K.K.: J. Chromatogr. B 688, 303 (1997).
4. Mann B., Grayeski M.L.: Biomed. Chromatogr. 1991, 5, 47 (1991).
5. Zhang G., Terry A.V. Jr, Bartlett M.G.: J. Chromatogr. B 856, 20 (2007).
6. Gutteck U., Rentsch K.M.: Clin. Chem. Lab. Med. 41, 1571 (2003).
7. Kratzsch C., Peters F.T., Kraemer T., Weber A.A., Maurer H.H.: J. Mass Spectrom. 38, 283 (2003).
8. Hattori H., Yamamoto S., Iwata M., Takashima E., Yamada T., Suzuki O.: J. Chromatogr. 579, 247 (1992).
9. Pujadas M., Pichini S., Civit E., Santamariña E., Perez K., de la Torre R.: J. Pharm. Biomed. Anal. 44, 594 (2007).
10. Maślanka A., Krzek J.: J. AOAC Int. 88, 70 (2005).
11. Higashi Y., Nakamura S., Fujii Y.: Biomed. Chromatogr. 20, 964 (2006).
12. Titier K., Bouchet S., Péhourcq F., Moore N., Molimard M.: J. Chromatogr. B 788, 179 (2003).
13. Zhang G., Terry A.V. Jr, Bartlett M.G.: J. Chromatogr. B 858, 276 (2007).
14. Mennickent S., Pino L., Vega M., de Diego M.: J. Sep. Sci. 31, 201 (2008).
15. Mennickent S., Pino L., Vega M., Godoy C.G., de Diego M.: J. Sep. Sci. 30, 77 (2007).

Received: 15. 12. 2008